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EFFECTS OF ANTITOXIDANTS ON RESISTANCE TO RADIATION INJURY

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FOREWORD

The investigations described in this report were carried out during the period of 15 March 1962 to 30 January 1963, under Project No. 7164, "Space Biology Research," and Task No. 716405, "Nutrition in Aerospace Flight." The research was supported by Contract No. AF 33(657)-8401 with the Biomedical Laboratory, 6570th Aerospace Medical Research Laboratories, Aerospace Medical Division, Wright-Patterson Air Force Base, Ohio. The project was initiated by 1st Lt. E.G. Sander and was monitored by 1st Lt. P.A. Lachance, Biospecialties Section, Physiology Branch, Biomedical Laboratory. The technical assistance of Mr. C.W. Steers, Jr., L. Slaughter, M. Pleasant, L. Galpern, and Mrs. J. Leynnwood of the Western Biological Laboratories is gratefully acknowledged. The radiation facilities were designed and constructed by the Isotopes Specialties Company, Burbank, California, to whom we are indebted for the maintenance and monitoring of the radiation facilities. Animal experimentation was conducted in accordance with the Principles of Laboratory Animal Care as established by the National Society for Medical Research.

ABSTRACT

Since BHT (butylated hydroxy toluene) and certain other antioxidants significantly prolonged the average survival time of mice exposed to multiple sublethal doses of total body x-irradiation, experiments were undertaken to determine the effects of antioxidants of diverse structure and activity and antioxidant synergists on length of survival following continuous exposure to a lethal dose of total body gamma radiation in the rat. BHT when fed at a 0.5% level in a purified diet and ascorbic acid at a 0.1% level significantly increased the average survival time of irradiated rats over that of rats fed the unsupplemented purified diet. Polygard (tri(nonylated phenyl) phosphites) when fed at levels of 0.25% and 0.5% and citric acid and phosphoric acid at levels of 0.1% in the purified diet also appeared to have some activity in this regard. Other antioxidants tested had little if any protective effect. The average survival time of irradiated rats was significantly longer for rats fed a natural food stock ration than for those fed the basal purified diet. No correlation was observed between the weight increment, organ weights or microscopic appearance of the tissues of irradiated rats with length of survival on any of the diets employed.

PUBLICATION REVIEW

This technical documentary report has been reviewed and is approved.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

A. Introduction

Available data indicate that the deleterious effects of ionizing radiation are due, at least in part, to the formation of chemically active free radicals and the oxidation of various biological compounds by such radicals (Stein and Weiss (ref. 20) and Barron (ref. 3)). If such were the case, preventing or inhibiting the oxidation reactions due to free radical activity might be expected to have a protective effect. Attempts to counteract radiation injury by administering tocopherol, a naturally occurring antioxidant, however, have been unsuccessful. Furth et al. (ref. 10) administered alpha-tocopherol in oil to rats prior to irradiation without beneficially improving survival. Haley et al. (ref. 12) found that intramuscular injections of a water-soluble vitamin E to mice immediately prior to x-irradiation were similarly without protective effect. On the contrary large doses of vitamin E in this species significantly decreased survival following exposure to a lethal dose of total body x-irradiation. Since antioxidants may differ in respect to their concentration and distribution in the tissues after administration as well as in their capacity to inhibit oxidative reactions under physiological conditions, studies were conducted on the effects of antioxidants of diverse structure and activity on length of survival following exposure to multiple sublethal doses of total body x-irradiation in the mouse (Ershoff and Steers (ref. 9)). Significant differences were obtained with different antioxidants. Whereas mixed tocopherols and Santoquin (6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline) at a 0.25% level in the diet and DPPD (N,N'-diphenyl-p-phenylenediamine) at levels of 0.25% or 0.5% in the diet had little, if any, effect on length of survival, the day on which 50% mortality was attained or the number of animals surviving as compared to results on the basal unsupplemented diet, propyl gallate, DBH (2,5-di-tert-butyl-hydroquinone) and BHT (butylated hydroxy toluene) at levels of 0.25% or 0.5% of the diet prolonged survival over that of animals fed the unsupplemented basal ration. Propyl gallate administered 30 minutes before x-irradiation was also active in reducing mortality following exposure to a minimal lethal dose of x-irradiation (600 r) in mice (Gorodetskii et al. (ref. 11)). A protective effect against symptoms of radiation injury has also been obtained on occasion with ascorbic acid (Loiseleur and Velley (ref. 15)), another substance with antioxidant activity, but results with this compound have been variable (Bacq and Herve (ref. 2)). It is of interest that many of the radioprotective agents such as reducing agents and chelating agents also have antioxidant properties (Alexander et al. (ref. 1)). The following experiment was conducted to determine the effects of antioxidants of diverse structure and activity and compounds which are antioxidant synergists (i.e., ascorbic acid, citric

acid, and phosphoric acid) when fed alone and with antioxidants on length of survival following continuous exposure to a lethal dose of total body gamma radiation in the rat.

B. Experimental

Seven hundred and two male rats of the Holtzman strain were selected at an average body weight of 48.6 gm (range 42 to 54 gm) and were divided into 26 comparable groups of 27 animals each. These were placed in metal cages with raised screen bottoms (3 rats per cage) and were provided the various diets indicated in Table 1 and distilled water ad libitum. Animals were fed 4 times weekly (Monday, Wednesday, Friday and Saturday) and all food not consumed by the next feeding was discarded. The basal purified diet employed in these studies consisted of sucrose, 67.7%; Vitamin-Free Test Casein, 22%; Wesson Salt Mixture, 5%; cottonseed oil, 5%; l-cystine, 0.3%; and the following vitamins per kg of diet: thiamine hydrochloride, 10 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 10 mg; calcium pantothenate, 60 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; biotin, 1 mg; folic acid, 10 mg; para-aminobenzoic acid, 200 mg; inositol, 400 mg; vitamin B_{12}, 150 µg; 2-methyl,1-4 naphthoquinone, 5 mg; choline chloride, 2 gm; vitamin A, 5000 U.S.P. units; vitamin D_2, 500 U.S.P. units; and alpha-tocopherol acetate, 100 mg. The vitamins were added in place of an equal amount of sucrose. Tests were also conducted with rats fed a natural food stock ration (Rockland Complete Rat Diet in meal form).

After 12 days of feeding, each of the dietary groups* was subdivided into two groups consisting of 6 rats and 21 rats each. The smaller group served as non-irradiated controls; the larger group was exposed to total body gamma radiation from a Cesium-137/Barium-137 source at a level of 500 ± 10 r per week. Animals were continuously exposed to radiation except for short periods (20 to 30 minutes, 4 times per week) when they were fed, watered, and weighed. Cages were rotated 3 times weekly in an effort to provide uniform exposure for rats in the various groups, with individual cages being rotated not only from one side of the field to the other, but also from the highest position in the field to the lowest. After 7 weeks of radiation, 4 rats in each irradiated group (whose average body

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*In addition to the dietary groups listed in Table 1, experiments were also started with rats fed the basal purified diet + 0.25% and 0.5% DBH (2,5,di-tert-butyl-hydroquinone). DBH when incorporated at the above levels in the purified diet proved to be highly toxic with 51 of the 54 rats on the above diets succumbing within the first 12 days of feeding.

Table 1

Experimental Diets.*

1.	Basal Purified Diet
2.	Basal Purified Diet + 0.25% BHT (butylated hydroxy toluene)
3.	Basal Purified Diet + 0.50% BHT (butylated hydroxy toluene)
4.	Basal Purified Diet + 0.25% propyl gallate
5.	Basal Purified Diet + 0.50% propyl gallate
6.	Basal Purified Diet + 0.25% gum guaiac
7.	Basal Purified Diet + 0.50% gum guaiac
8.	Basal Purified Diet + 0.25% DL-TDP (B,B' dilauryl thiodipropionate)
9.	Basal Purified Diet + 0.50% DL-TDP (B,B' dilauryl thiodipropionate)
10.	Basal Purified Diet + 0.25% mixed tocopherols
11.	Basal Purified Diet + 0.50% mixed tocopherols
12.	Basal Purified Diet + 0.25% NDGA (nordihydroguaiacetic acid)
13.	Basal Purified Diet + 0.50% NDGA (nordihydroguaiacetic acid)
14.	Basal Purified Diet + 2% rutin
15.	Basal Purified Diet + 4% rutin
16.	Basal Purified Diet + 0.167% BHT (butylated hydroxy toluene), 0.167% DBH (2,5,di-tert-butyl-hydroquinone) and 0.167% propyl gallate
17.	Basal Purified Diet + 0.1% citric acid
18.	Basal Purified Diet + 0.1% ascorbic acid
19.	Basal Purified Diet + 0.1% phosphoric acid
20.	Basal Purified Diet + 0.033% citric acid, 0.033% ascorbic acid, and 0.033% phosphoric acid
21.	Basal Purified Diet + 0.167% BHT, 0.167% DBH, 0.167% propyl gallate, 0.033% citric acid, 0.033% ascorbic acid, and 0.033% phosphoric acid
22.	Basal Purified Diet + 0.25% enteric coated Polygard (tri(nonylated phenyl) phosphites)
23.	Basal Purified Diet + 0.50% enteric coated Polygard (tri(nonylated phenyl) phosphites)
24.	Rockland Complete Rat Diet in meal form**
25.	Rockland Complete Rat Diet in meal form + 0.167% BHT, 0.167% DBH, and 0.167% propyl gallate
26.	Rockland Complete Rat Diet in meal form + 0.167% BHT, 0.167% DBH, 0.167% propyl gallate, 0.033% citric acid, 0.033% ascorbic acid, and 0.033% phosphoric acid

*Test supplements were obtained from the following sources: BHT from Nopco Chemical Co., Richmond, Calif.; propyl gallate from Heyden Newport Chemical Corp., New York, N.Y.; gum guaiac from Hathaway Allied Products, Los Angeles, Calif.; DL-TDP from Halby Products Co., Wilmington, Del.; Mixed Tocopherols Concentrate N.F. and DBH from Distillation Products Industries, Rochester, N.Y.; NDGA from Wm. J. Strange Co., Oakland, Calif.; Polygard from Naugatuck Chemical, Division of United States Rubber Co., Naugatuck, Conn. The Polygard was enteric coated by S.O. Barnes & Son., Inc., Gardena, Calif.

**Rockland Complete Rat Diet, A.E. Staley Manufacturing Co., Decatur, Ill.

weight was comparable to that of the remaining animals in the group) and all rats in the non-irradiated groups were sacrificed and organ weights were determined. Tissues were placed in fixative and sections were prepared for microscopic examination. The experiment was terminated after 90 days of radiation, and the number of animals surviving and the average survival time of irradiated rats in the various dietary groups were determined.

C. Results

1. Growth

The body weight of rats in the various groups during the course of the experiment is indicated in Table 2. Findings indicate that on each of the diets employed the weight increment of irradiated rats was significantly less than that of non-irradiated rats on the same diet. In the irradiated series rats gained weight during the first four to five weeks of radiation and thereafter lost weight; in the non-irradiated series they gained weight throughout the experimental period. No significant differences were observed in the relative growth retardation (i.e., the ratio of body weight of irradiated to non-irradiated rats) in the various dietary groups resulting from radiation exposure.

2. Organ Weights

Data on the kidney, testes, seminal vesicles, thymus, spleen and adrenal weights of rats sacrificed after the 7th week of radiation in both the irradiated and non-irradiated series are summarized in Table 3. A highly significant reduction in the weight of the testes, seminal vesicles, thymus and spleen and a marked increment in the ratio of adrenal to body weight occurred in all irradiated rats compared to that of non-irradiated rats in the same dietary groups. No significant differences were observed between the various dietary groups in respect to organ weight changes induced by radiation exposure.

3. Survival

Data on the average survival time of irradiated rats in the various groups are summarized in Table 4. Findings indicate that there was considerable heterogenicity in the various treatment groups. The experiment was terminated after the 90th day of radiation and animals alive at that time were arbitrarily assigned a value of 90 days survival time. Since only 1 rat survived in the group fed the unsupplemented purified diet in contrast to 4 or more in some of the other groups, differences may have been even more marked had the experiment continued longer. This consideration is particularly pertinent in the

Table 2

Body weights during experiment

Dietary Group	Average body weight at start of radiation period	Average body weight after following weeks of radiation: 2	4	7
		gm	gm	gm
Irradiated series				
1	116.8	151.3 (20)	181.0 (20)	172.9 (13)
2	120.2	147.1	180.4	171.3 (16)
3	116.9	140.1 (20)	167.6 (20)	167.2 (19)
4	115.0	149.0	182.2	168.8 (17)
5	110.9	138.9 (20)	163.9 (20)	167.4 (16)
6	116.8	149.2	180.5	166.4 (17)
7	111.4	139.6 (20)	163.0 (20)	157.1 (15)
8	117.8	152.3	186.3	170.5 (17)
9	120.2	142.0	178.1	169.6 (14)
10	113.4	152.3	191.9	172.5 (12)
11	120.4	151.7 (20)	190.3 (20)	172.3 (13)
12	115.1	132.1 (20)	159.0 (20)	155.7 (16)
13	101.3	122.8 (19)	145.7 (19)	149.1 (13)
14	112.2	142.0	168.0	156.8 (17)
15	104.6	140.7	177.3	177.7 (16)
16	94.3	125.9 (20)	149.5 (20)	149.7 (15)
17	113.5	145.4	182.3	178.6 (19)
18	112.4	149.7 (18)	176.0 (18)	179.1 (15)
19	112.7	147.2	185.7 (20)	172.9 (17)
20	116.5	150.6	184.9 (20)	173.5 (17)
21	97.4	125.8 (19)	147.3 (19)	146.5 (15)
22	119.7	153.9	181.9	180.9 (16)
23	121.4	154.6	189.9 (20)	177.2 (17)
24	120.4	156.5	189.5	169.2 (20)
25	110.1	142.4	166.8 (20)	178.8 (18)
26	109.9	138.3	164.2	176.4 (18)
Non-irradiated series				
1	120.3	207.5	279.8	341.2
2	119.2	186.3	263.3	325.3
3	116.7	186.8	262.2	337.2
4	113.3	192.3	264.8	323.0
5	109.5	190.0	258.8	315.3
6	113.7	186.7	254.3	312.5
7	114.0	184.5	256.5	328.0 (5)
8	115.7	199.5	269.2	349.6
9	115.8	186.2	264.0	322.2
10	116.3	194.8	261.7	327.8
11	115.5	189.8	254.3	332.4 (5)
12	113.8	186.7	257.2	324.7
13	111.8	193.5	266.2	316.0
14	126.0	204.8	275.0	335.3
15	131.2	209.8	284.3	348.7
16	89.3	165.5	237.5	297.3
17	115.7	194.2	268.6	337.4
18	116.7	203.2	279.2	349.8
19	114.5	187.3	250.2	323.4
20	115.7	199.0	269.5	327.6
21	98.8	159.5	232.8	288.4
22	119.2	202.2	271.8	333.4
23	114.7	187.2	250.6	325.5
24	121.7	206.3	282.0	351.6
25	103.0	195.8	281.0	364.8
26	113.8	185.0	268.8	336.4

Originally 21 animals were employed in each irradiated group and 6 in each non-irradiated group. The values in parentheses indicate the number of animals alive at the time data were obtained when this number was less than the original number in the group.

Table 3

Organ weights of irradiated and non-irradiated rats*.

Dietary Group	Total body wt.	Kidneys	Testes	Seminal Vesicles	Thymus	Spleen	Adrenals	Ratio of adrenal wt. to body wt.
	gm	gm	gm	mg	mg	mg	mg	mg per 100 gm
Irradiated series								
1	174.5	1.75	.675	242	111	167	43.0	24.6
2	181.0	1.72	.849	265	129	154	47.7	26.4
3	177.5	1.77	.900	233	124	196	48.7	27.4
4	166.7	1.57	.925	192	120	216	45.3	27.2
5	173.5	1.77	.950	189	123	202	32.3	18.6
6	175.8	1.88	.844	239	120	197	38.0	21.6
7	173.5	1.60	.850	227	144	199	41.0	23.6
8	178.0	1.98	.701	202	122	242	36.5	20.5
9	179.3	1.75	.800	247	139	217	38.8	21.6
10	183.5	1.80	.825	256	88	218	44.0	24.0
11	169.5	1.77	.850	249	85	173	46.0	27.1
12	164.2	1.60	.854	182	106	159	47.0	28.6
13	163.0	1.62	.800	252	97	201	39.5	24.2
14	161.3	1.53	.804	196	82	242	41.0	25.4
15	169.5	1.60	.850	212	75	202	48.0	28.3
16	167.3	1.55	.660	176	84	169	36.0	21.5
17	178.0	1.85	.875	236	99	204	44.8	25.2
18	165.8	1.67	.750	142	106	191	43.5	26.2
19	173.5	1.70	.729	229	93	172	40.5	23.3
20	190.3	1.73	.825	289	130	215	42.3	22.2
21	165.5	1.60	.750	196	103	184	38.0	23.0
22	167.7	1.80	.741	242	108	177	39.7	23.7
23	177.0	1.70	.734	182	101	158	42.5	23.9
24	183.7	1.65	.900	222	106	179	41.7	22.7
25	186.5	1.60	.717	303	115	189	39.0	20.9
26	186.0	1.62	.925	183	375	205	42.0	22.6
Non-irradiated series								
1	341.2	2.90	3.32	950	667	900	36.5	10.7
2	325.3	2.72	3.42	775	600	775	33.0	10.1
3	337.2	3.27	3.32	1.000	725	1.000	47.0	13.9
4	323.0	2.70	3.42	825	668	975	39.7	12.3
5	315.3	2.75	3.32	737	725	1.05	45.5	14.4
6	312.5	3.10	3.20	837	637	875	39.7	12.7
7	328.0	2.97	3.32	775	692	1.000	41.0	12.5
8	349.6	3.17	3.55	825	560	850	40.7	11.6
9	322.2	2.90	3.50	700	725	737	41.7	12.9
10	327.8	3.20	3.30	987	800	987	44.5	13.6
11	332.4	3.27	3.55	912	728	925	40.5	12.2
12	324.7	3.17	3.35	770	775	900	38.2	11.8
13	316.0	3.25	3.30	775	725	950	45.7	14.5
14	335.3	3.05	3.42	850	582	850	43.0	12.8
15	348.7	3.20	3.30	725	703	950	40.2	11.5
16	297.3	2.72	3.30	875	445	850	42.7	14.4
17	337.4	2.90	3.32	825	552	787	35.6	10.6
18	349.8	3.08	3.47	775	665	800	42.0	12.0
19	323.4	2.70	3.20	875	646	850	41.7	12.9
20	327.6	2.67	3.40	700	496	800	45.5	13.9
21	288.4	2.70	3.05	686	588	800	39.5	13.7
22	333.4	3.00	3.17	850	750	900	40.0	12.0
23	325.0	3.15	2.77	775	590	825	40.0	12.3
24	351.6	2.85	3.58	850	645	950	43.5	12.4
25	364.8	3.15	3.98	800	668	1.10	44.2	12.1
26	336.4	2.75	3.75	602	425	900	44.2	13.1

Data on irradiated rats were based on 4 animals per group; those on non-irradiated rats on 6 animals per group except for the groups fed diets 7 and 11 which consisted of 5 rats each.

*Animals were sacrificed after the 7th week of radiation.

Table 4

Average survival time of irradiated rats*.

Dietary Group	Survival time in days		Total mean radiation dose**	Number of rats surviving***
	Mean	Stand. dev.		
1	54.44	12.748	3888.59	1
2	61.06	19.239	4361.45	2
3	69.12	15.903	4937.17	3
4	59.94	14.745	4281.45	2
5	62.25	18.336	4446.46	1
6	56.76	9.704	4054.31	0
7	53.50	10.058	3821.45	0
8	54.94	12.660	3924.31	0
9	54.41	11.874	3886.45	1
10	51.24	11.075	3660.02	1
11	52.81	12.062	3772.17	1
12	57.81	11.451	4129.31	1
13	51.87	9.978	3705.02	0
14	56.41	12.664	4029.31	0
15	60.00	12.072	4285.74	0
16	55.69	12.810	3977.88	1
17	68.05	18.675	4861.46	5
18	69.50	15.388	4964.32	1
19	64.81	15.021	4629.31	2
20	59.75	13.331	4267.88	1
21	60.36	17.030	4311.45	2
22	63.53	18.880	4537.88	4
23	64.82	18.750	4630.03	5
24	63.94	10.958	4567.17	1
25	67.31	16.268	4807.89	2
26	64.41	15.704	4600.74	3

*Animals that succumbed during the first 4 weeks of radiation were not included in the tabulation of data.

**Computed on the basis of 71.429 r per day.

***The experiment was terminated after 90 days of radiation. Data were calculated on the basis of a 90 day survival time for animals alive at the termination of the experiment.

case of rats fed the Polygard and citric acid supplements (diets 22, 23, and 17). Considering the above, a nonparametric analysis seemed to be indicated. Thus the median test (Siegel (ref. 19)) was used to determine whether irradiated rats in the various dietary groups differed in respect to length of survival when compared to animals fed the basal purified diet. This approach required that the median survival time (in days) be found for each analysis. The number of animals above and below the median in each group were then compared with the animals fed the basal ration and significance was evaluated by using chi square. These analyses are summarized in Table 5.

In agreement with earlier findings on mice exposed to multiple sublethal doses of total body x-irradiation (Ershoff and Steers (ref. 9)), BHT (butylated hydroxy toluene) when fed at a 0.5% level in the diet significantly increased as indicated by the chi square test the average survival time of gamma irradiated rats over that of animals fed the basal purified diet alone. Polygard (tri(nonylated phenyl) phosphites) at levels of 0.25% and 0.5% of the diet also appeared to have some activity in this regard but the differences observed did not quite attain statistical significance. Other antioxidants tested (i.e., propyl gallate, gum guaiac, DL-TDP (B,B' dilauryl thiodipropionate), mixed tocopherols and NDGA (nordihydroguaiacetic acid)) at levels of 0.25% and 0.5% of the diet and rutin at levels of 2% and 4% of the diet had little if any protective effect. The antioxidant synergists (citric acid, ascorbic acid, and phosphoric acid) when incorporated at a 0.1% level in the basal purified diet also resulted in a longer survival time than was obtained on the basal purified diet alone but only in the case of ascorbic acid were differences statistically significant. In agreement with previous findings (Ershoff and Graham (ref. 8)), the average survival time of rats continuously exposed to 500 r per week total body gamma irradiation was also significantly longer for animals fed a natural food stock ration (Rockland Complete Rat Diet in meal form) than for those fed the basal purified diet. The increased survival time on the various diets indicated above occurred despite the fact that animals in these groups received more total radiation than did rats fed the unsupplemented basal diet. This situation obtained because animals were continuously irradiated as the experiment progressed and hence the rats which survived were exposed to a greater total dose of radiation than were those which succumbed earlier.

4. Histological Findings

Irradiated rats in all dietary groups exhibited the following morphological changes which were not observed in any of the non-irradiated rats in any of the dietary groups:

Bone. The findings of the femur and tibia were essentially comparable to those observed by Heller (ref. 13), Liebow et al.

Table 5

Chi square analyses of frequency of animals above and below median survival time.

A. Diet 1 vs diets 2 and 3.

Diet	Median = 56 days Below	Above	Total
1	11	5	16
2	11	6	17
3	3	13	16
All Groups	25	24	49

χ^2 = 9.9544 df = 2 p < .01

B. Diet 1 vs diets 4 and 5

Diet	Median = 53 days Below	Above	Total
1	10	6	16
4	8	9	17
5	6	10	16
All Groups	24	25	49

χ^2 = 2.1216 df = 2 p > .05

C. Diet 1 vs diets 6 and 7

Diet	Median = 52 days Below	Above	Total
1	9	7	16
6	7	10	17
7	8	8	16
All Groups	24	25	49

χ^2 = .3760 df = 2 p > .05

Table 5 (continued)

D. Diet 1 vs diets 8 and 9

Diet	Median = 52 days Below	Above	Total
1	9	7	16
8	8	9	17
9	8	9	17
All Groups	25	25	50

χ^2 = .3676 df = 2 p $\geq$.05

E. Diet 1 vs diets 10 and 11

Diet	Median = 49 days Below	Above	Total
1	8	8	16
10	9	8	17
11	8	8	16
All Groups	25	24	49

χ^2 = .6819 df = 2 p $\geq$.05

F. Diet 1 vs diets 12 and 13

Diet	Median = 53 days Below	Above	Total
1	8	8	16
12	6	10	16
13	8	7	15
All Groups	22	25	47

χ^2 = .8797 df = 2 p $\geq$.05

Table 5 (continued)

G. Diet 1 vs diets 14 and 15

Diet	Median = 53 days Below	Above	Total
1	10	6	16
14	10	7	17
15	5	12	17
All Groups	25	25	50

χ^2 = 4.4118 df = 2 p $\geq$ .05

H. Diet 1 vs diet 16

Diet	Median = 51 days Below	Above	Total
1	9	7	16
16	8	8	16
All Groups	17	15	32

χ^2 = .1254 df = 1 p $\geq$ .05

I. Diet 1 vs diet 17

Diet	Median = 54 days Below	Above	Total
1	10	6	16
17	8	9	17
All Groups	18	15	33

χ^2 = .7894 df = 1 p $\geq$ .05

Table 5 (continued)

J. Diet 1 vs diet 18

Diet	Median = 59 days Below	Above	Total
1	11	5	16
18	3	11	14
All Groups	14	16	30

χ^2 = 6.7773 df = 1 p < .01

K. Diet 1 vs diet 19

Diet	Median = 56 days Below	Above	Total
1	11	5	16
19	6	10	16
All Groups	17	15	32

χ^2 = 3.1372 df = 1 p > .05

L. Diet 1 vs diet 20

Diet	Median = 54 days Below	Above	Total
1	10	6	16
20	7	9	16
All Groups	17	15	32

χ^2 = 1.1294 df = 1 p > .05

Table 5 (continued)

M. Diet 1 vs diet 21

Diet	Median = 52 days Below	Above	Total
1	9	7	16
21	7	8	15
All Groups	16	15	31

χ^2 = .2834 df = 1 p $\geq$.05

N. Diet 1 vs diets 22 and 23

Diet	Median = 56 days Below	Above	Total
1	10	5	15
22	8	9	17
23	7	9	16
All Groups	25	23	48

χ^2 = 1.8945 df = 2 p $\geq$.05

O. Diet 1 vs diet 24

Diet	Median = 56 days Below	Above	Total
1	11	5	16
24	4	13	17
All Groups	15	18	33

χ^2 = 6.8083 df = 1 p $\leq$.01

Table 5 (continued)

P. Diet 1 vs diet 25

Diet	Median = 56 days Below	Above	Total
1	11	5	16
25	4	12	16
All Groups	15	17	32

χ^2 = 6.1490 df = 1 p $\leq$ .02

Q. Diet 1 vs diet 26

Diet	Median = 55 days Below	Above	Total
1	11	5	16
26	6	11	17
All Groups	17	16	33

χ^2 = 3.7003 df = 1 p $\geq$ .05

(ref. 14), Rust et al. (ref. 18), and Warren (ref. 21). The changes were marked by degeneration of the epiphyseal cartilage, decreased interdigitation of the cartilage at the epiphyseal line, "severance" of the spongiosa bone from the epiphyseal cartilage (Fig. 1), disappearance of osteocytes and osteoblasts, atypical metaphyseal cortical bone, cessation of growth, and permanent stunting.

Bone Marrow. The hemopoietic tissue damage was similar to that described by Bloom (ref. 4), Dunlap (ref. 7), Rust et al. (ref. 18) and Warren (ref. 21) and was indicated by degeneration and depletion of the marrow which was reduced to a very loose syncitial reticulum containing cell debris, gelatinous marrow, adipose tissue and macrophages (Fig. 2). Sinusoidal engorgement, and megakaryocytic reduction were pronounced. Attempt at hemopoietic regeneration was evidenced by foci of small dark-staining cells especially in the epiphyseal and metaphyseal spaces.

Lymph nodes. All the nodes examined had a "washed out" appearance due to necrosis and depletion of the lymphocytes, and obliteration of the germinal centers (Figs. 3 & 4). Most nodes were reduced to a mere reticular syncitium containing a few lymphocytes, hemosiderin deposits, macrophages, plasma cells, mast cells, and serous exudate. Severe lymphocytic depletion resulted in enormous dilatation of the sinusoids and subcapsular spaces which were usually filled with macrophages, most of which had engulfed blood pigment and cell debris (Fig. 5). These changes were consistent with those reported by De Bruyn (ref. 5), Dunlap (ref. 7), and Warren (ref. 21).

Spleen. Damage in the spleen was principally in the white pulp which showed changes closely similar to those of the lymph nodes (Fig. 6). In addition, erythropoiesis was completely absent and reticulo-endothelial cells were prominent. These observations were in accord with those of Murray (ref. 16), Liebow et al. (ref. 14), Rust et al. (ref. 18), and Warren (ref. 21).

Testes. Testicular morphologic changes were among the most striking finding and were characterized by atrophy, intratubular and intertubular edema and complete aspermatogenesis in all tubules due to absolute absence of spermatogonia, spermatids, and spermatocytes, and most of the Sertoli cells (Fig. 7). Comparable changes have been reported by Liebow et al. (ref. 15), Murphree et al. (ref. 17), Rust et al. (ref. 18) and Warren (ref. 21). Sterility was permanent and complete due to absolute destruction of germinal epithelium and eventual absence of regeneration.

Intestines. The major findings observed were regression and obliteration of the focal lymph nodules.

Teeth. The dental lesions were observed in practically all areas of the tooth excepting the enamel and were basically (a) pyknosis, collagenolysis and vascular canalization of the periodontium (Fig. 8), (b) periodontal cell inclusions in the acellular cementum and dentin (Fig. 9), (c) ectopic dentification and hemosiderin deposits in the pulp (Fig. 10), (d) gingivitis (Fig. 11), and (e) interdental pyorrhea (Fig. 12).

Other Tissues. The changes in other tissues studied were inconsistent and non-specific except for hemosiderin deposits in the reticulo-endothelial cells.

No significant differences were observed in respect to the pathological changes induced by radiation exposure in the tissues of rats in the various dietary groups.

5. General Observations

No correlation was observed between the weight increment, organ weights or microscopic appearance of the tissues of irradiated rats with length of survival on the various diets employed.

D. Discussion

Most studies on radioprotective agents have been conducted with animals exposed to a single lethal dose of total body radiation. From a practical point of view, however, this situation does not simulate that which is likely to occur under field conditions. If radioprotective agents are to be employed by man, they are most likely to be used under conditions where exposure to radiation is continuous for prolonged periods of time (for example, radiation induced by atomic weapons or radiation in a space environment). For those exposed to a massive dose of radiation (for example, from an atomic blast or a solar flare), the degree of protection that might be afforded by radioprotective chemicals is negligible. At a distance from an atomic blast or in a space environment, however, it is possible that radioprotective agents may have a significant protective effect. The cause of death in animals exposed to fractionated or continuous radiation is frequently different from that of animals which die after a single acute exposure (in which intestinal and bone marrow defects predominate). Hence, agents which protect against the latter may be ineffective against the former, and conversely agents that might have a significant protective effect in the chronically irradiated animal may be without protective effect in those exposed to a single lethal dose. Thus, Doull et al. (ref. 6) reported that 2-mercaptoethylamine (MEA), 5-hydroxytryptamine (5-HT), 2-aminoethylisothiourea (AET), p-aminopropiophenome (PAPP) and diethyldithiocarbamic acid (DEDT)

were ineffective in prolonging the survival time of chronically x-irradiated mice at dosage levels adequate to reduce acute radiation lethality. Conversely, ascorbic acid under conditions of the present experiment had a significant protective effect in contrast to its relative inactivity under conditions of acute x-irradiation (Bacq and Herve (ref. 2)). Further studies are indicated to determine the modus operandi of the latter effect and the effects of other antioxidants and/or antioxidant synergists thereon.

E. Summary

Immature male rats were fed a highly purified diet and similar diets containing antioxidants of diverse structure and activity and/or compounds which were antioxidant synergists. After 12 days of ad libitum feeding each dietary group was divided into two subgroups, one of which served as non-irradiated controls while the other was continuously exposed to 500 r per week total body gamma radiation from a Cesium-137/Barium-137 source for 90 days or until death, whichever occurred sooner. BHT (butylated hydroxy toluene) when fed at a 0.5% level in the diet significantly increased the average survival time of gamma irradiated rats over that of animals fed the basal purified diet alone. Polygard (tri(nonylated phenyl) phosphites) at levels of 0.25% and 0.5% of the diet also appeared to have some activity in this regard but the differences observed did not quite attain statistical significance. Other antioxidants tested (i.e., propyl gallate, gum guaiac, DL-TDP (B,B' dilauryl thiodipropionate), mixed tocopherols and NDGA (nordihydroguaiacetic acid)) at levels of 0.25% and 0.5% of the diet and rutin at levels of 2% and 4% of the diet had little if any protective effect. The antioxidant synergists (citric acid, ascorbic acid, and phosphoric acid) when incorporated at a 0.1% level in the basal purified diet also resulted in a longer survival time than was obtained on the basal purified diet alone but only in the case of ascorbic acid were differences statistically significant. The average survival time of irradiated rats was also significantly longer for animals fed a natural food stock ration than for those on the unsupplemented purified diet. No correlation was observed between the weight increment, organ weights, or microscopic appearance of the tissues of irradiated rats with length of survival on any of the diets employed.

FIGURES

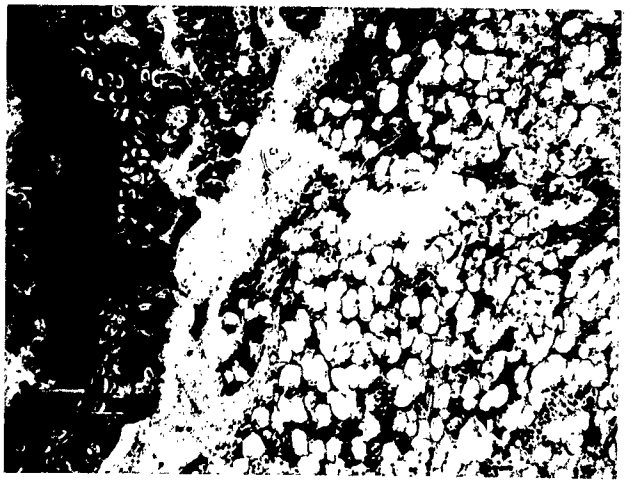

Fig. 1. "Severance" of spongiosa bone from epiphysial cartilage, absence of spongy bone and depletion of hemopoietic tissue in the metaphysis of a tibia. H & E stain x 105.

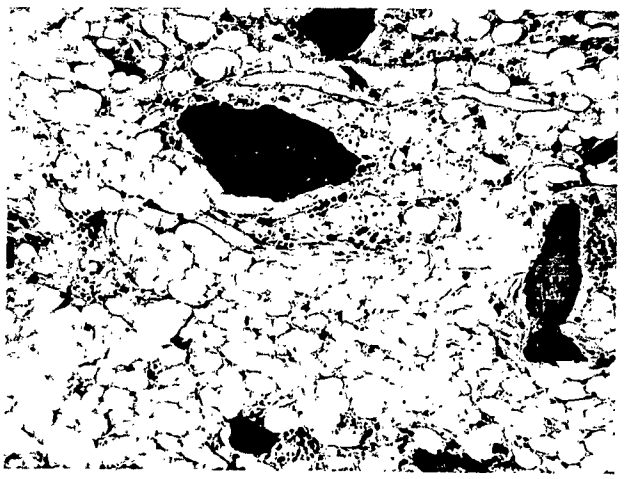

Fig. 2. Metaphyseal bone marrow in a tibia. Note loose syncitial marrow containing cell debris, gelatinous marrow, adipose tissue, dilated sinusoids, and prominent reticulo-endothelial cells; also disappearance of osteoblasts and megakaryocytes; and virtual absence of hemopoietic tissue. H & E stain x 105.

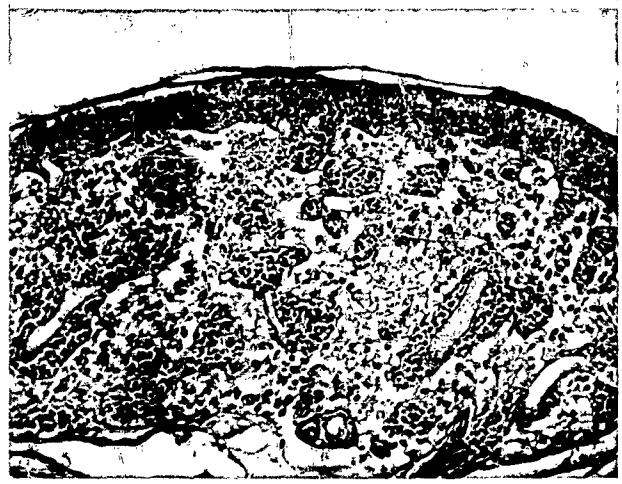

Fig. 3. Mandibular lymph node showing marked lymphocytic depletion, obliteration of germinal centers, filling of the subcapsular space with macrophages, and capillary engorgement. H & E stain x 105.

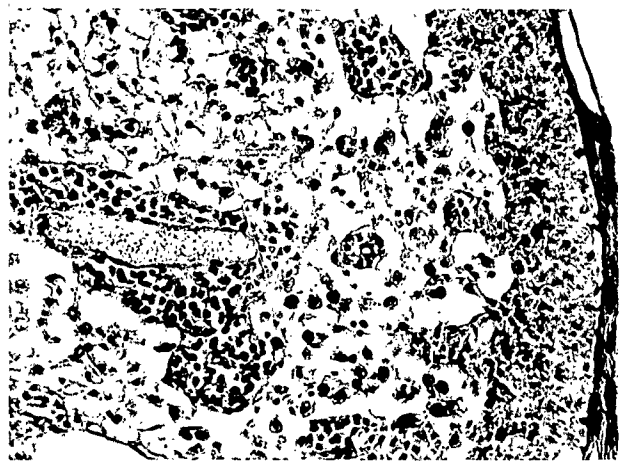

Fig. 4. Same as in Fig. 3. Note syncitial appearance of the cortex, and dark-staining mast cells. H & E stain x 210.

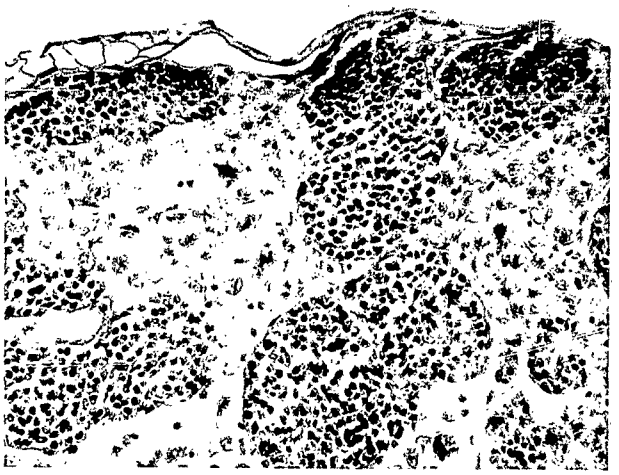

Fig. 5. Macrophages filling the dilated sinusoids of a mandibular lymph node. H & E stain x 210.

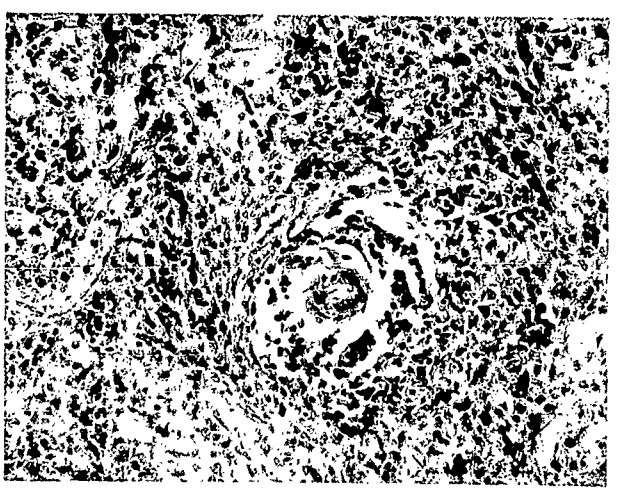

Fig. 6. Spleen with typical lymphocytic depletion and necrosis of a germinal center, and prominent reticulo-endothelial cells. H & stain x 210.

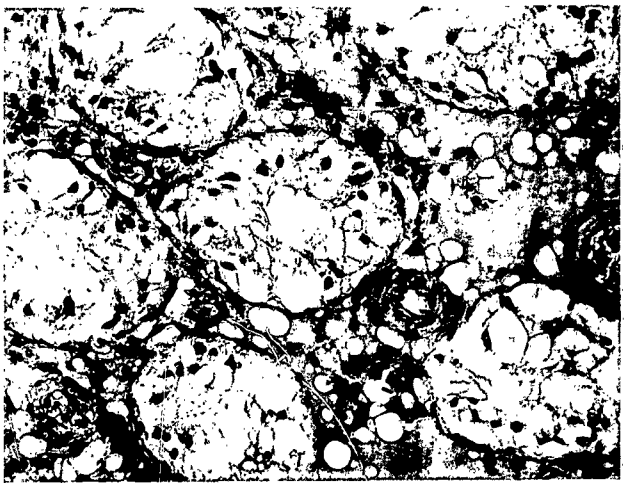

Fig. 7. Typical aspermatogenic testis. Note virtual absence of spermatogonia, spermatids, spermatocytes, most of the Sertoli cells. Tubular atrophy and intertubular edema are also pronounced. H & E stain x 210.

Fig. 8. Pyknosis, collagenolysis, and vascular canalization of the periodontium of a first molar. H & E stain x 210.

Fig. 9. Periodontal invasion of the cementum and dentin of a third molar. H & E stain x 210.

Fig. 10. Ectopic dentification (dark staining focal material) in the pulp of a second molar. H & E stain x 210.

Fig. 11. Gingivitis adjacent to a first molar. H & E stain x 210.

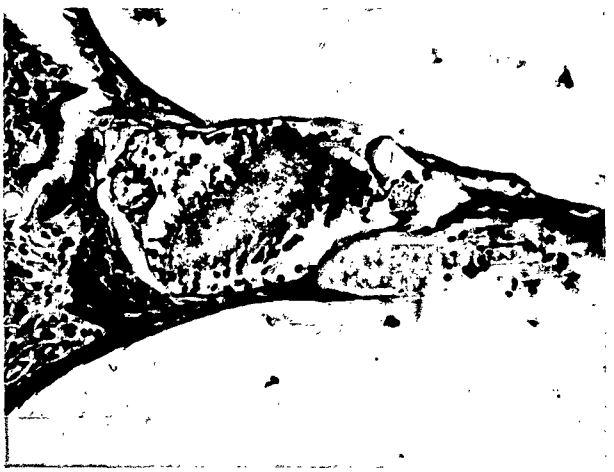

Fig. 12. Interdental pyorrhea between the first and second molar. Note a bacterial colony surrounded by heterophils. H & E stain x 210.

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UNCLASSIFIED

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2. Radiation Effects
3. Survival Time
4. Rats
5. Antioxidants
6. Toluenes

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II. Biomedical Laboratory
III. Contract AF 33 (616)-8401
IV. Western Biological Laboratories, Culver City, Calif.
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